SINGLE-STRANDED RNA-EDITING OLIGONUCLEOTIDES

RELATED APPLICATIONS

[0001] This application is a continuation of U.S. patent application Ser. No. 16/309,954, filed Dec. 14, 2018, which is a § 371 National Stage Application of PCT/EP2017/065467, filed Jun. 22, 2017, which claims priority to and the benefit of United Kingdom patent application No. 1610923.3, filed Jun. 22, 2016, United Kingdom patent application No. 1614669.8, filed Aug. 30, 2016, United Kingdom patent application No. 1702755.8, filed Feb. 21, 2017, and United Kingdom patent application No. 1706292.8, filed Apr. 20, 2017, the entire disclosures of each of which are incorporated herein by reference for all purposes.

FIELD OF THE INVENTION

[0002] The invention relates to the field of medicine. More in particular, it relates to the field of RNA editing, whereby an RNA sequence is targeted by a single-stranded antisense oligonucleotide to specifically correct a mutation in the RNA sequence.

BACKGROUND OF THE INVENTION

[0003] RNA editing is a natural process through which eukaryotic cells alter the sequence of their RNA molecules, often in a site-specific and precise way, thereby increasing the repertoire of genome encoded RNAs by several orders of magnitude. RNA editing enzymes have been described for eukaryotic species throughout the animal and plant kingdoms, and these processes play an important role in managing cellular homeostasis in metazoans from the simplest life forms (such as *Caenorhabditis elegans*) to humans. Examples of RNA editing are adenosine (A) to inosine (I) conversions and cytidine (C) to uridine (U) conversions, which occur through enzymes called adenosine deaminase and cytidine deaminase, respectively. The most extensively studied RNA editing system is the adenosine deaminase enzyme.

[0004] Adenosine deaminase is a multi-domain protein, comprising—depending on the enzyme in question—2 to 3 double-stranded RNA recognition domains and a catalytic domain. The recognition domain recognizes a specific double stranded RNA (dsRNA) sequence and/or conformation, whereas the catalytic domain converts an adenosine (A) into inosine (I) in a nearby, more or less predefined, position in the target RNA, by deamination of the nucleobase. Inosine is read as guanine by the translational machinery of the cell, meaning that, if an edited adenosine is in a coding region of an mRNA or pre-mRNA, it can recode the protein sequence.

[0005] A to I conversions may also occur in 5' non-coding sequences of a target mRNA, creating new translational start sites upstream of the original start site, which gives rise to N-terminally extended proteins, or in the 3' UTR or other non-coding parts of the transcript, which may affect the processing and/or stability of the RNA. In addition, A to I conversions may take place in splice elements in introns or exons in pre-mRNAs, thereby altering the pattern of splicing. As a consequence thereof, exons may be included or skipped. The adenosine deaminases are part of a family of enzymes referred to as Adenosine Deaminases acting on RNA (ADAR), including human deaminases hADAR1, hADAR2 and hADAR3.

[0006] The use of oligonucleotides to edit a target RNA applying adenosine deaminase has been described (e.g. Montiel-Gonzalez et al. PNAS 2013, 110(45):18285-18290; Vogel et al. 2014. Angewandte Chemie Int Ed 53:267-271; Woolf et al. 1995. PNAS 92:8298-8302). Montiel-Gonzalez et al. (2013) described the editing of a target RNA using a genetically engineered fusion protein, comprising an adenosine deaminase domain of the hADAR2 protein, fused to a bacteriophage lambda N protein, which recognises the boxB RNA hairpin sequence. The natural dsRNA binding domains of hADAR2 had been removed to eliminate the substrate recognition properties of the natural ADAR and replace it by the boxB recognition domain of lambda N-protein. The authors created an antisense oligonucleotide comprising a 'guide RNA' part that is complementary to the target sequence for editing, fused to a boxB portion for sequence specific recognition by the N-domain-deaminase fusion protein. By doing so, it was elegantly shown that the guide RNA oligonucleotide faithfully directed the adenosine deaminase fusion protein to the target site, resulting in guide RNA-directed site-specific A to I editing of the target RNA. These guide RNAs, disclosed in Montiel-Gonzalez et al. (2013), are longer than 50 nucleotides, which is generally too long for therapeutic applications (difficulties in manufacturing and cell entry). A disadvantage of this method in a therapeutic setting is also the need for a fusion protein consisting of the boxB recognition domain of bacteriophage lambda N-protein, genetically fused to the adenosine deaminase domain of a truncated natural ADAR protein. It requires target cells to be either transduced with the fusion protein, which is a major hurdle, or that target cells are transfected with a nucleic acid construct encoding the engineered adenosine deaminase fusion protein for expression. The latter requirement constitutes no minor obstacle when editing is to be achieved in a multicellular organism, such as in therapy against human disease to correct a genetic disorder.

[0007] Vogel et al. (2014) disclosed editing of RNA coding for eCFP and Factor V Leiden, using a benzylguanine substituted guide RNA and a genetically engineered fusion protein, comprising the adenosine deaminase domains of ADAR1 or 2 (lacking the dsRNA binding domains) genetically fused to a SNAP-tag domain (an engineered O6-alkylguanine-DNA-alkyl transferase). Although the genetically engineered artificial deaminase fusion protein could be targeted to a desired editing site in the target RNAs in HeLa cells in culture, through its SNAP-tag domain which is covalently linked to a guide RNA through a 5'-terminal O6-benzylguanine modification, this system suffers from similar drawbacks as the genetically engineered ADARs described by Montiel-Gonzalez et al. (2013), in that it is not clear how to apply the system without having to genetically modify the ADAR first and subsequently transfect or transduct the cells harboring the target RNA, to provide the cells with this genetically engineered protein. Clearly, this system is not readily adaptable for use in humans, e.g. in a therapeutic setting.

[0008] Woolf et al. (1995) disclosed a simpler approach, using relatively long single stranded antisense RNA oligonucleotides (25-52 nucleotides in length) wherein the longer oligonucleotides (34-mer and 52-mer) could promote edit-